Drug Resistance in Cancer: Mechanism and Management

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NIH National Institutes of Health

Drug Resistance in Cancer

- May reflect resistance to single agents generally by altering targets; resistance may arise from mutations in targets or by mutations that bypass targets
- Multidrug resistance affects all classes of drugs, including newly designed targeted drugs, and frequently results from alterations in mechanisms that detoxify drugs (e.g., uptake, metabolism, sequestration, efflux, etc.)
- Both single agent and multidrug resistance may also result from alterations in growth-promoting pathways, altered differentiation pathways (e.g., EMT), or different cells of origin

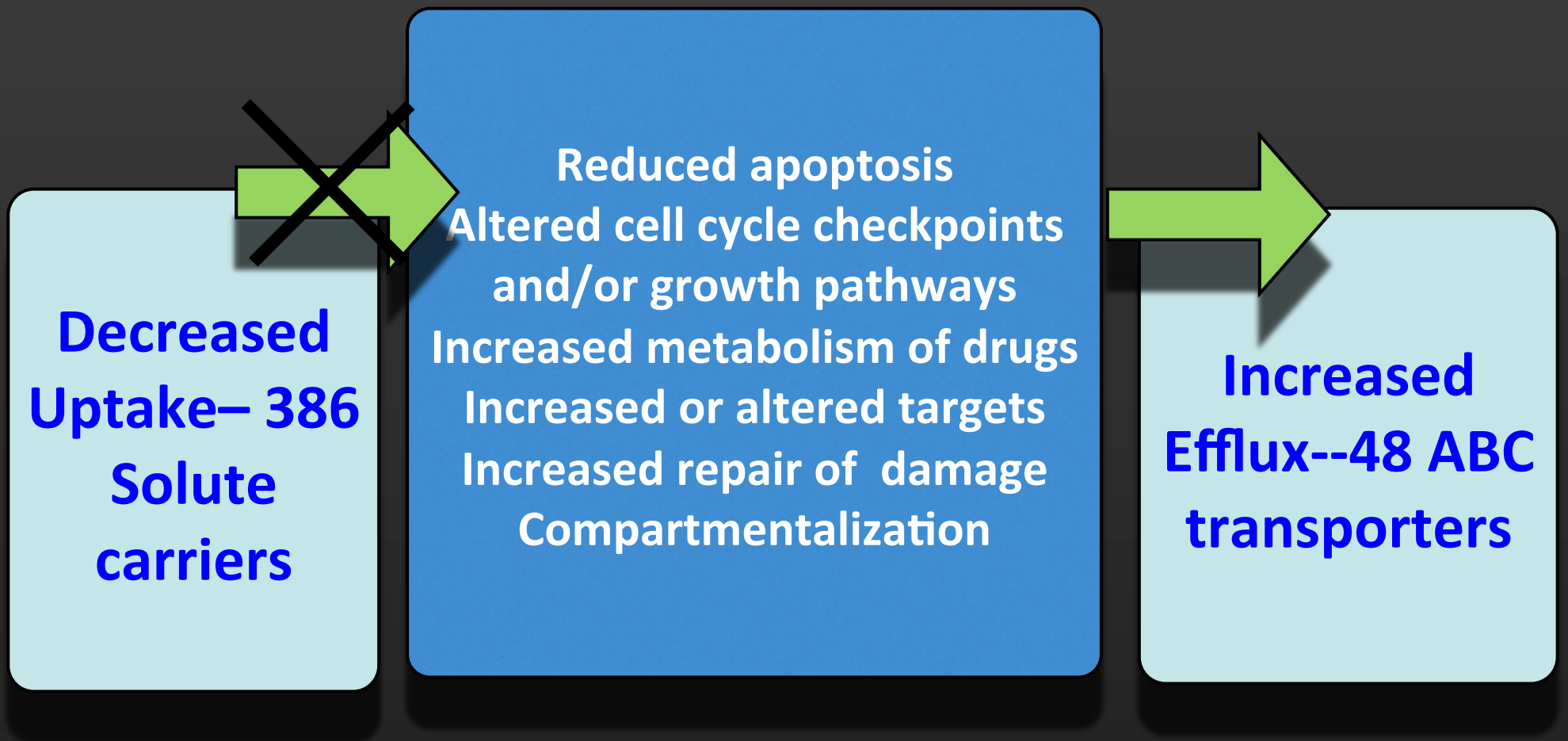
Factors that increase likelihood of drug resistance

- Heterogeneity of original cancer cell population
- Increased mutation rate or epigenetic change
- Inducibility of resistance mechanisms

Summary of Talk

- Role of ABC transporters in multidrug resistance in cancer and at the blood brain barrier
- Relevance of NCI-60 cell lines to the study of drug resistance in clinical cancer
- Complexity of MDR in 3 clinical cancers (ovarian cancer, hepatocellular carcinoma, and acute myelogenous leukemia)
- Models that account for clinical data

Mechanisms of resistance to anti-cancer drugs



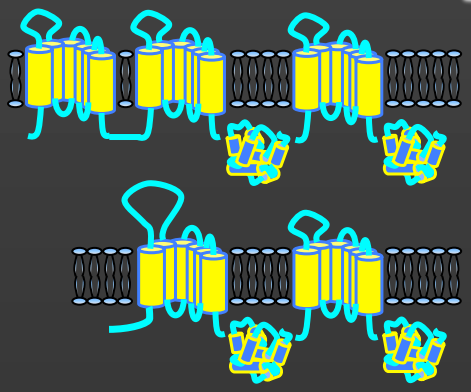
ATP-Binding Cassette (ABC) Transporter Superfamily

- One of the largest family of transport proteins known. Currently, more than 2000 members have been identified.

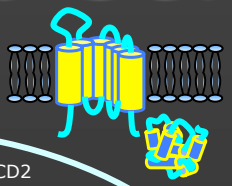
- Transport substrates include-- ions, sugars, glycans, phospholipids, cholesterol, peptides, proteins, toxins, antibiotics, and hydrophobic natural product anticancer drugs.

- Structurally, consist of various combinations of ATP-binding cassettes and segments with 6 trans-membrane domains.

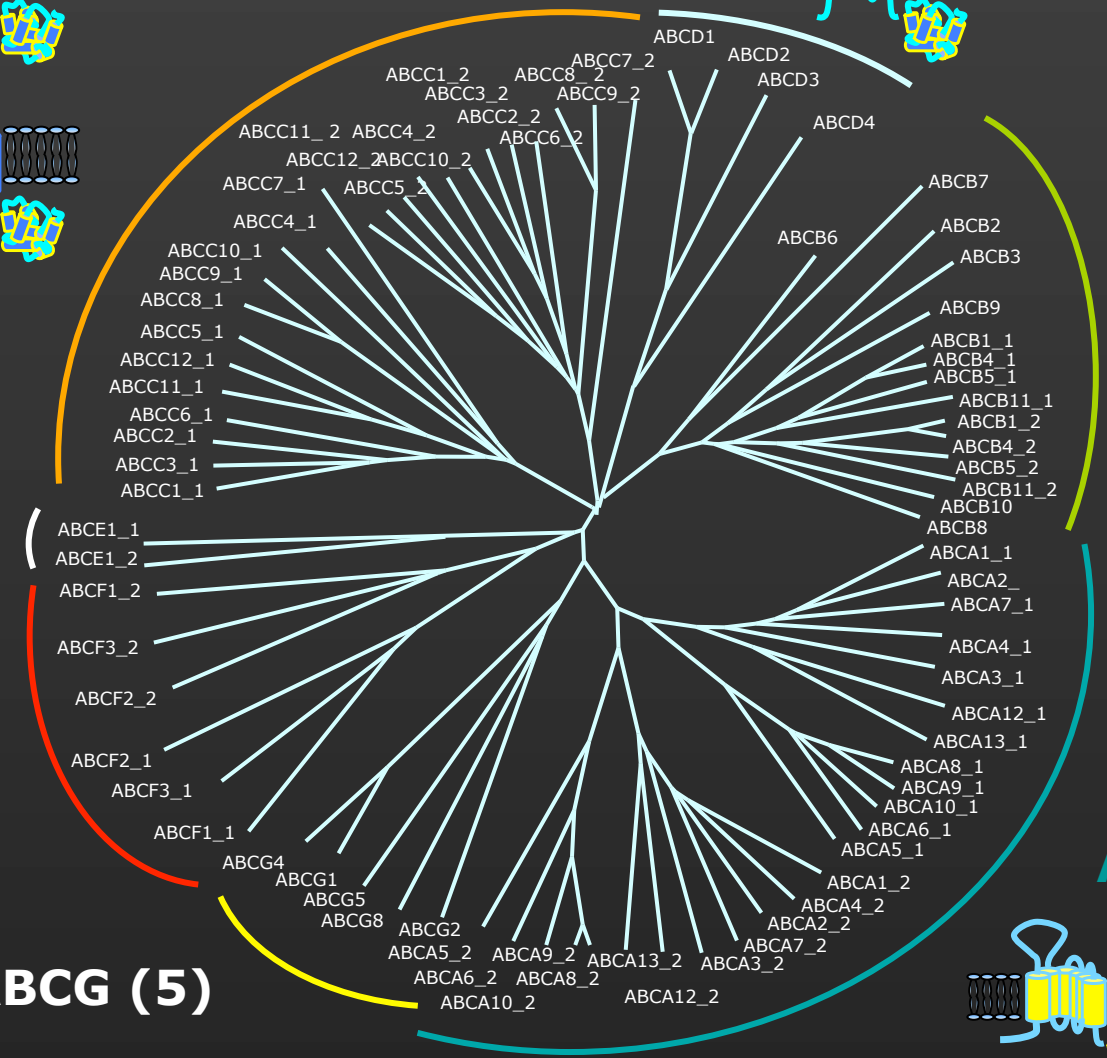
48 Human ABC Genes

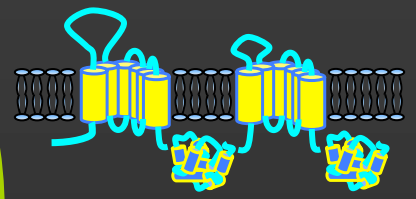


ABCC (12)

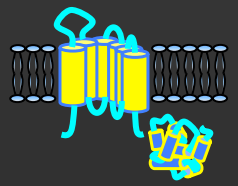


ABCD (4)





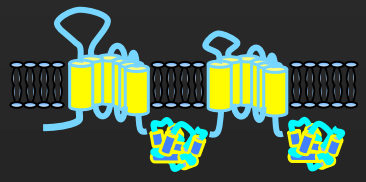
ABCB (11)



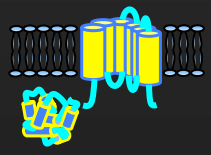
ABCE (1)

ABCF (3)

ABCA (12)

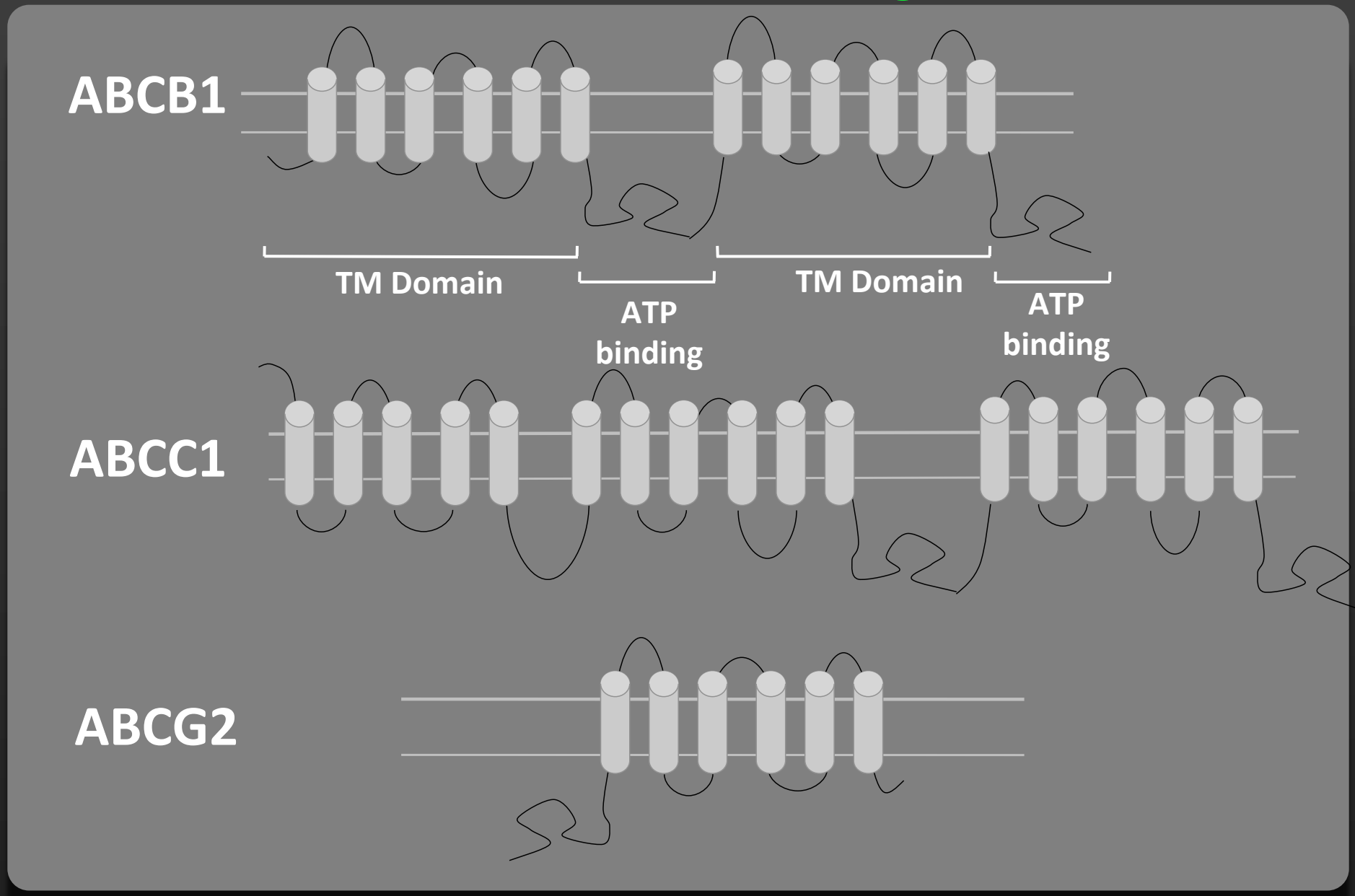


ABCG (5)

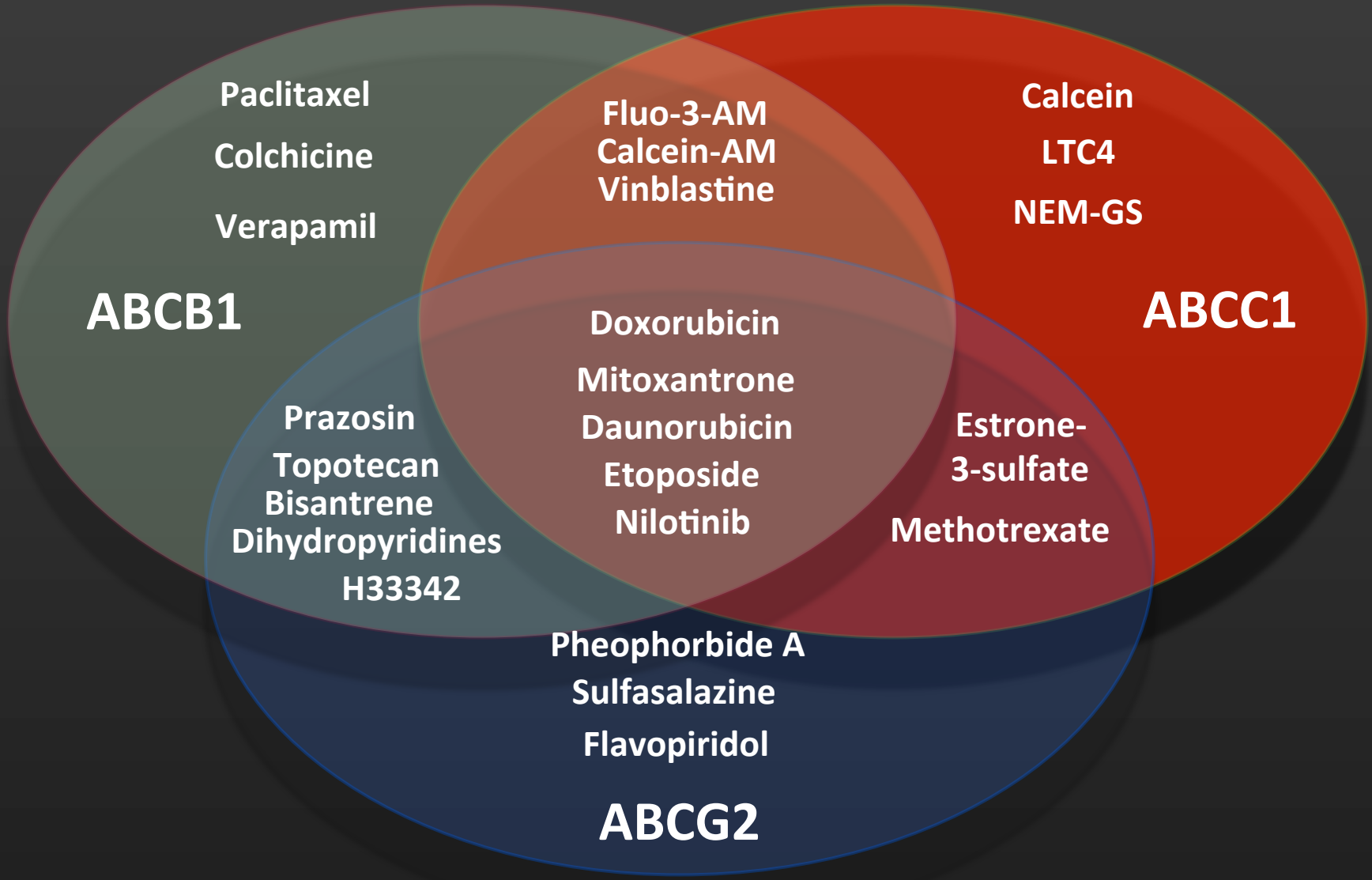


The Clustal W program was used to make the alignment of the NBDs and the tree was built by using the MEGA program -- By Mike Dean, NCI

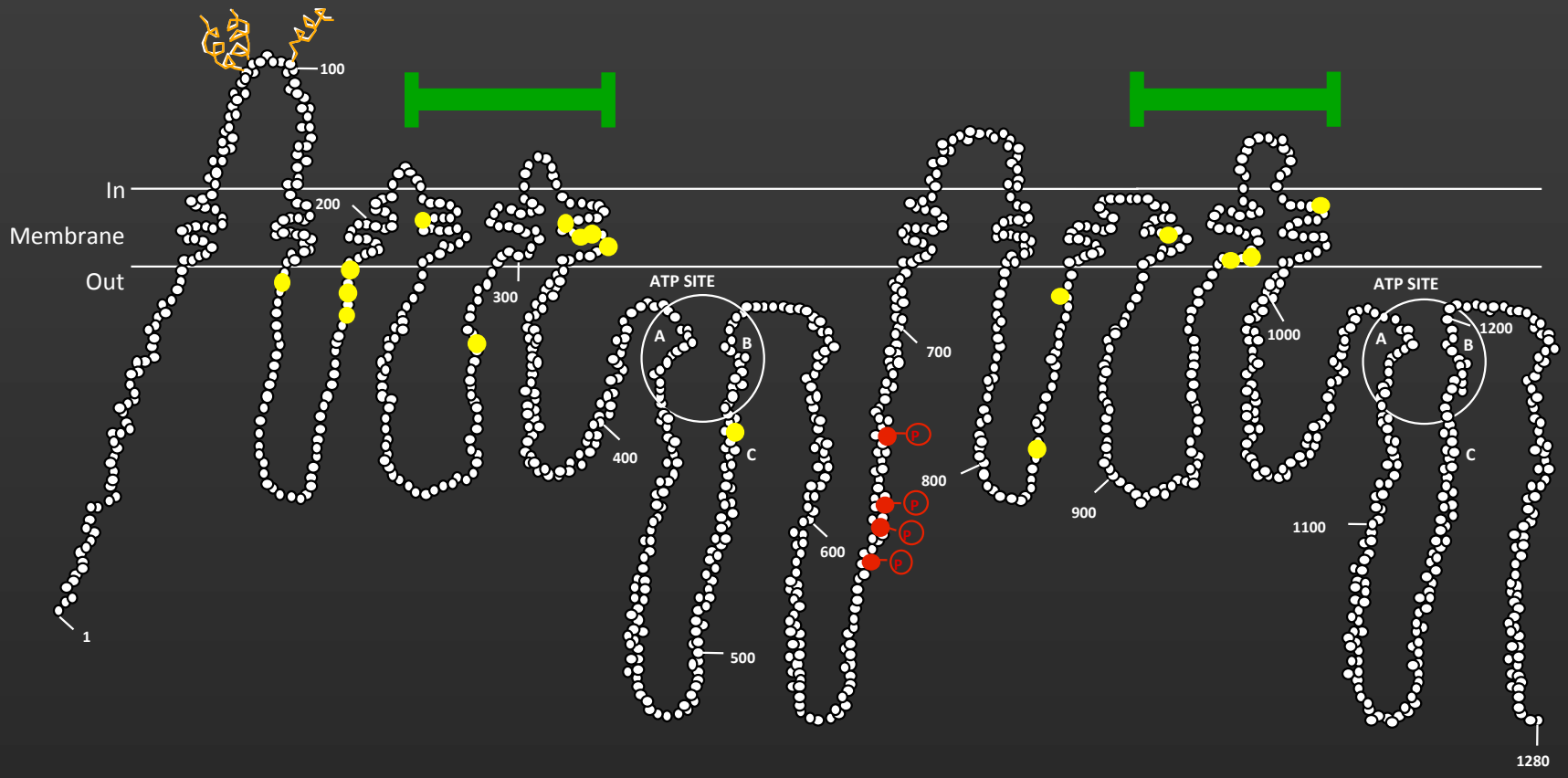
ABC transporters that form the blood-brain barrier and confer MDR: Domain organization



Overlapping substrate specificity of ABCB1, ABCG2 and ABCC1



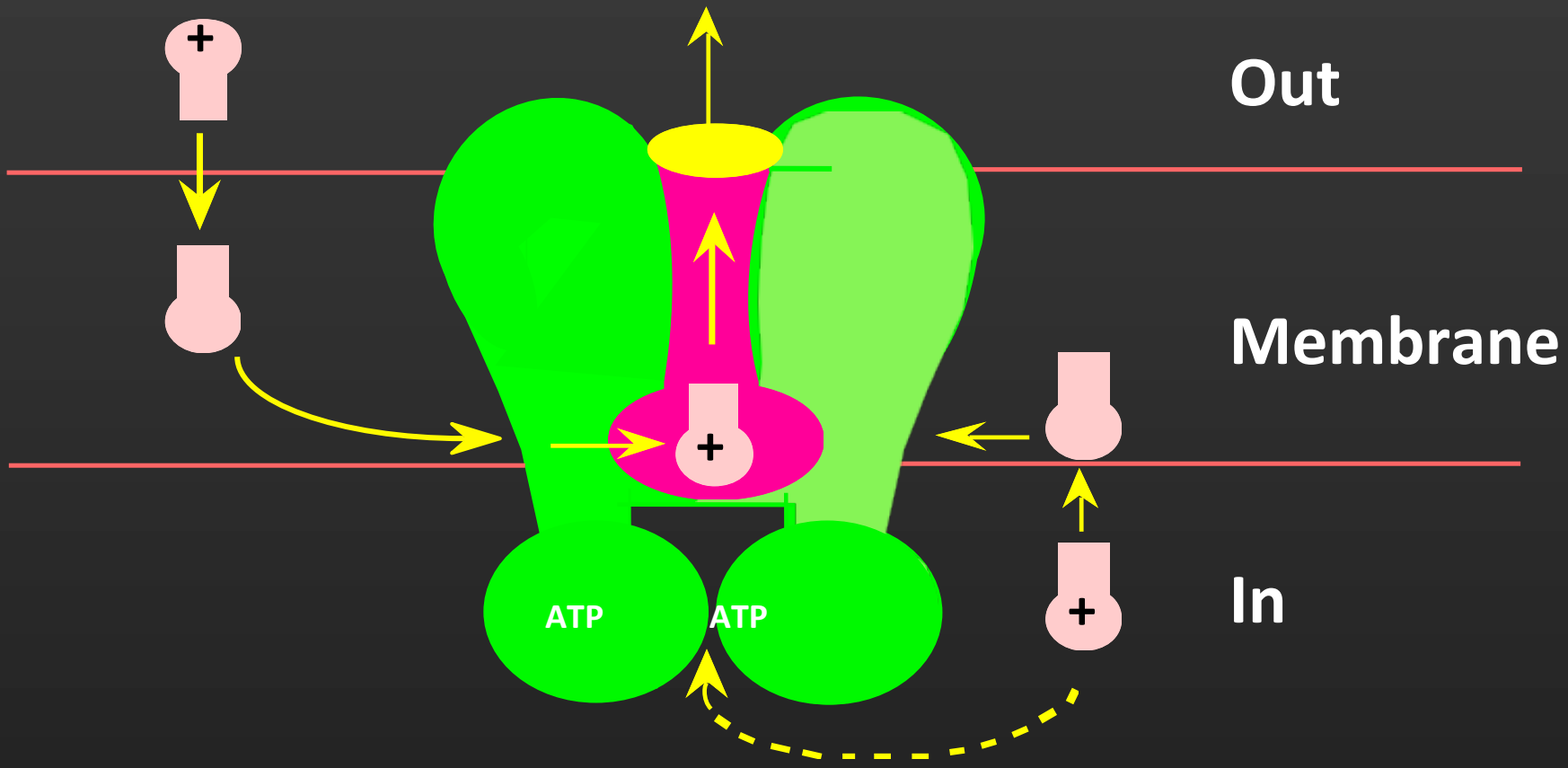
Hypothetical Model of Human P-glycoprotein



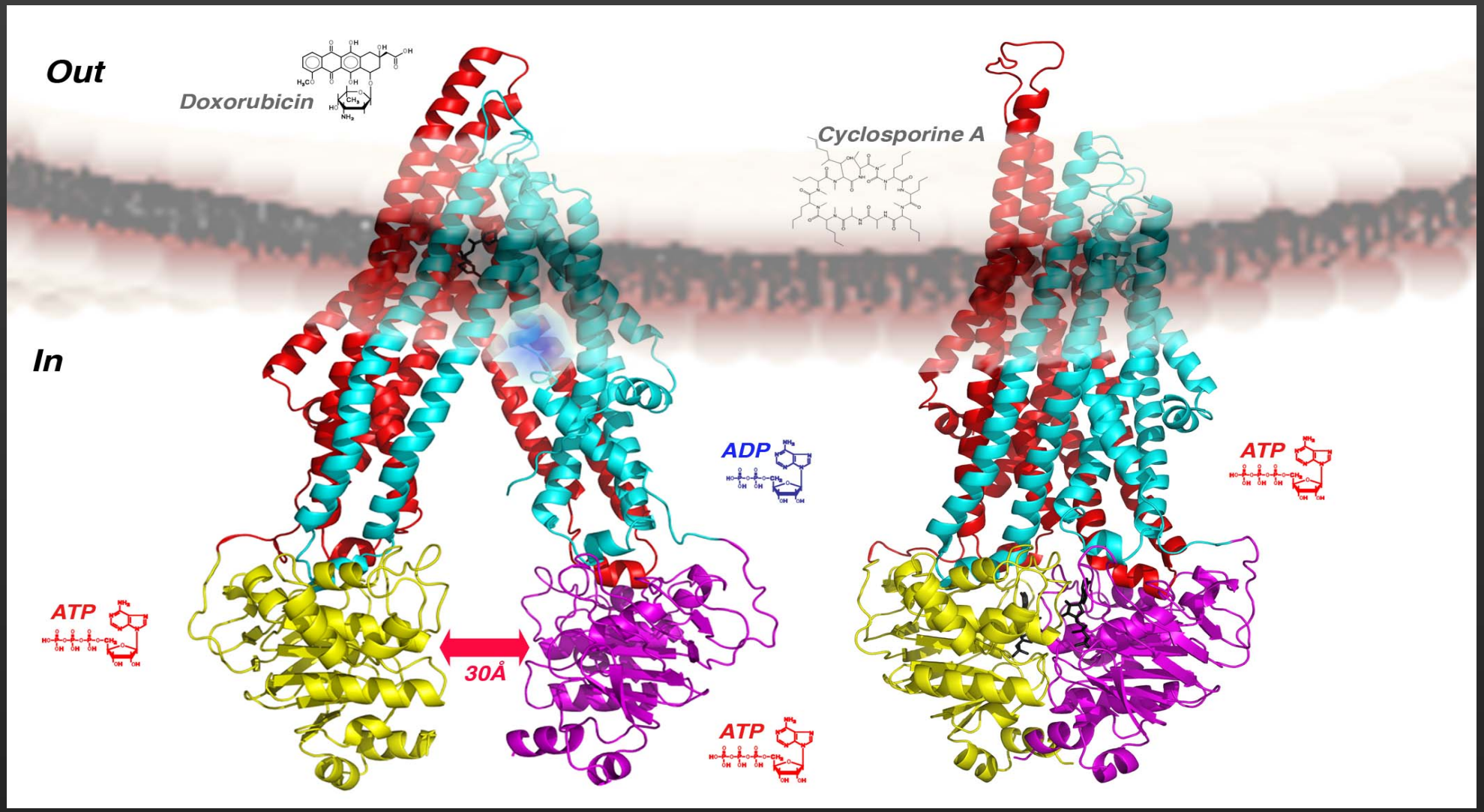
POINT MUTATIONS ( )

PHOTOAFFINITY LABELED REGIONS ( ), AND PHOSPHORYLATION SITES (P)

P-glycoprotein removes hydrophobic substrates directly from the plasma membrane



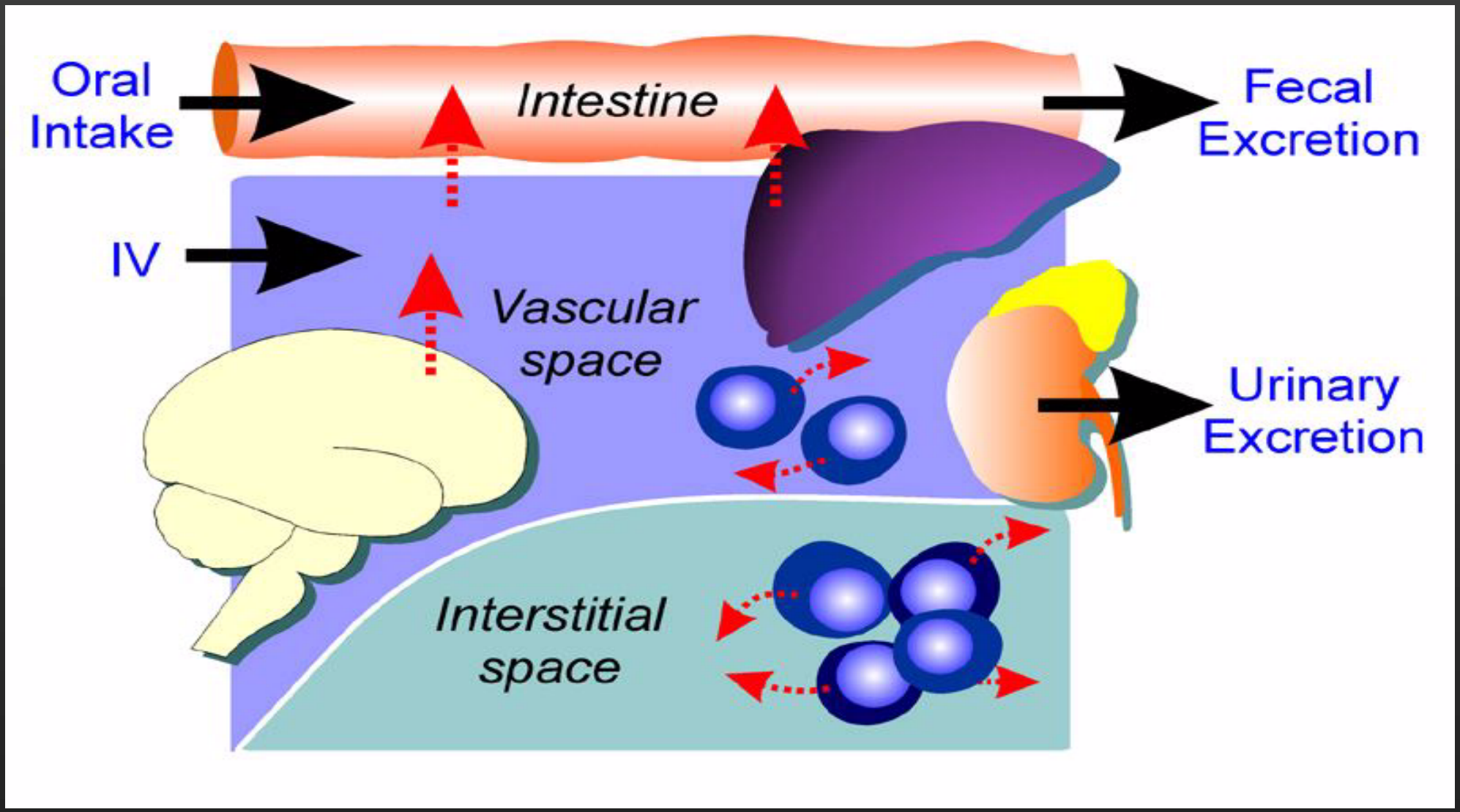
Atomic models of the structures of P-gp



Mouse P-gp at 3.8Å (Aller and Chang)

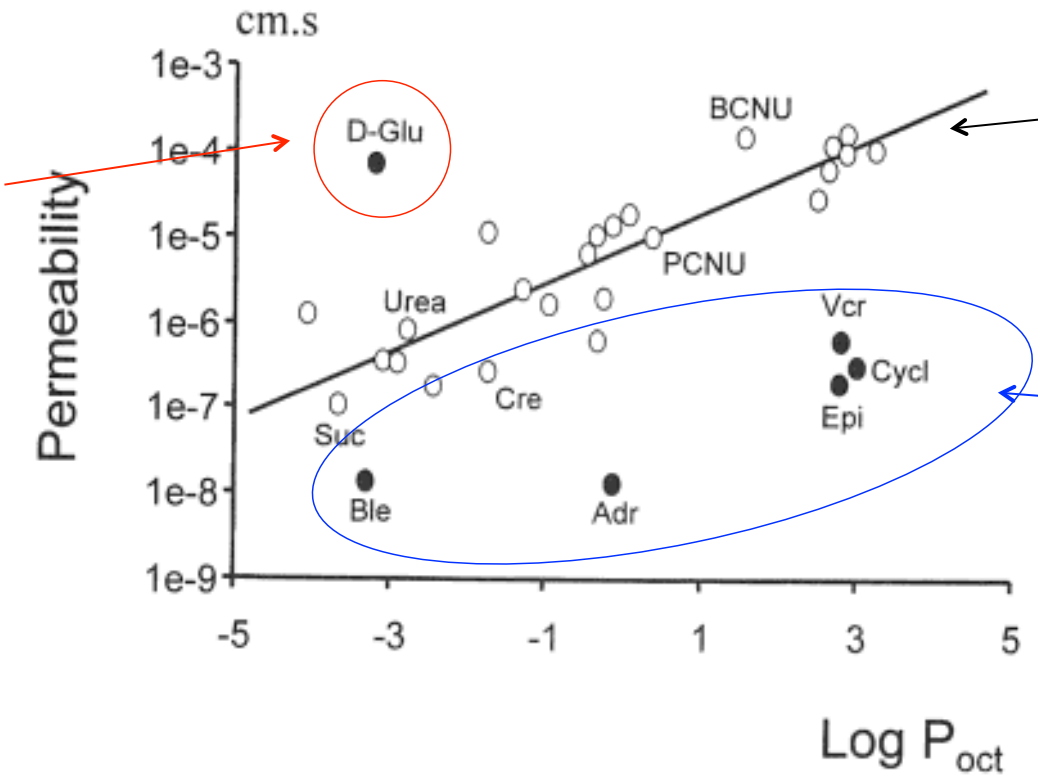
Human P-gp model based on Sav1866 (Xia)

Physiologic Role of P-glycoprotein



Many factors affect brain penetration – logP and transport

Active uptake



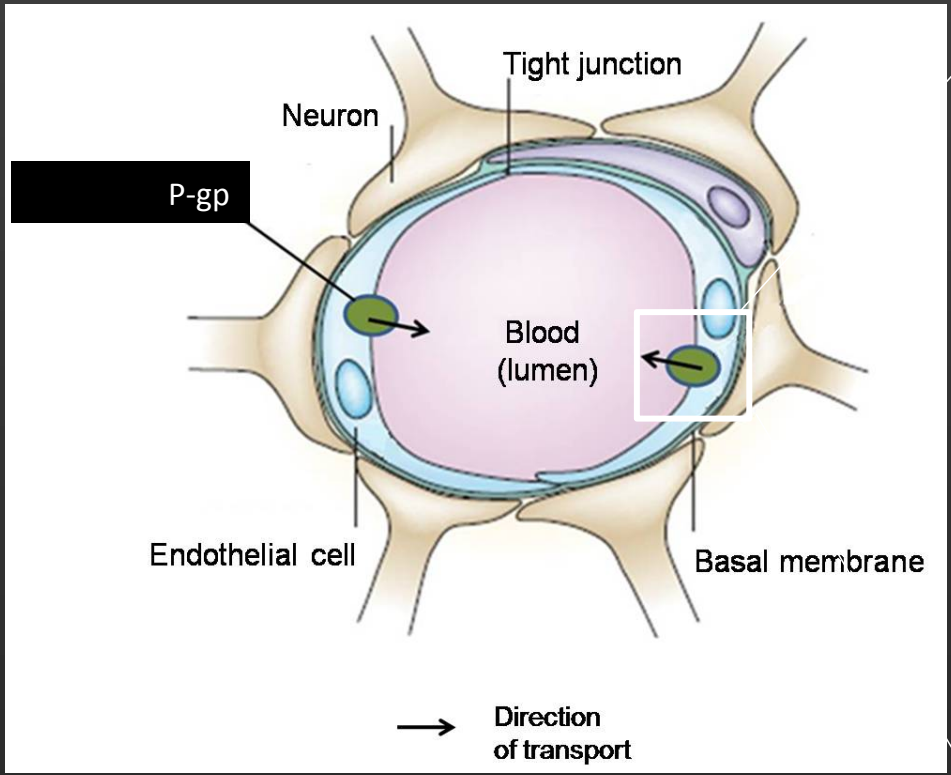
Passive diffusion

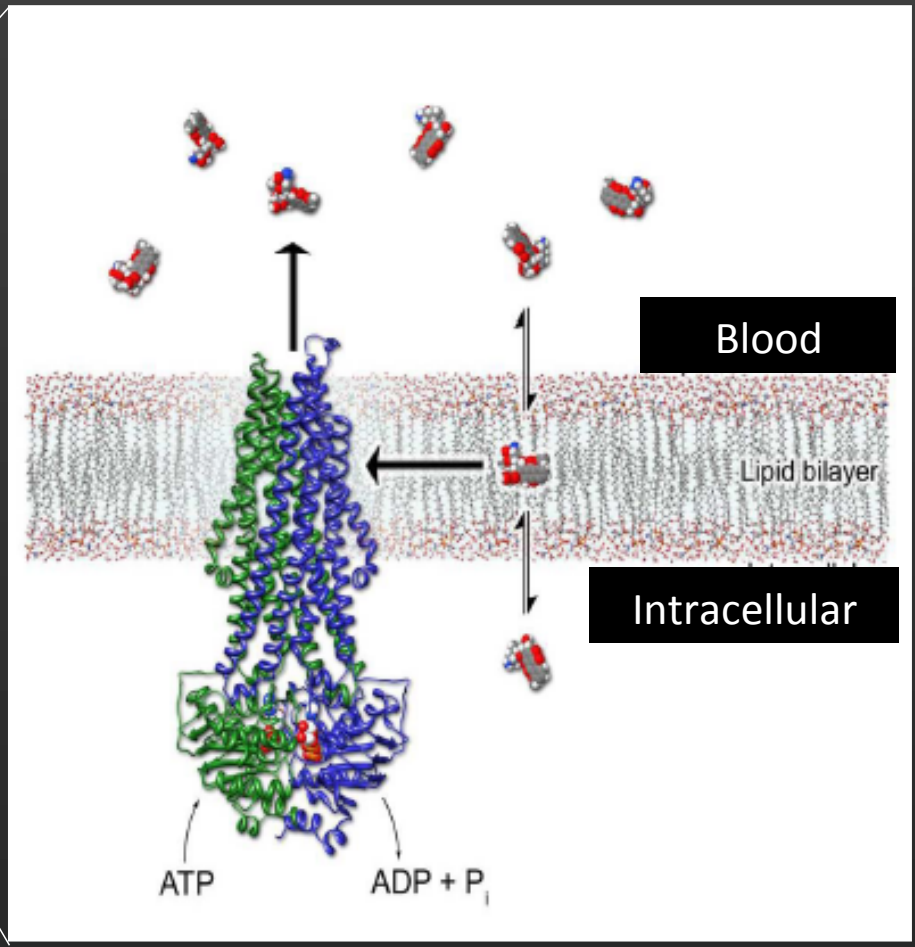
Active efflux

Fig. 1 Plot of CNS permeability against log $P_{octanol}$. Many solutes (open circles) show a clear correlation between their lipid solubility, determined as log P_{oct} and CNS penetration; Suc, sucrose; Cre, creatinine; PCNU, (1-(2-1-nitrosourea; BCNU, 1,3-bis-chloro(2-chloroethyl)1-nitrosourea. Solutes that show an enhanced or depressed uptake at the BBB in relation to their lipid solubility are distinguished as marked outliers on this type of plot (solid circles) and either have a facilitated penetration at the BBB such as D-Glu (D-glucose) or an active efflux from the CNS as in the case of Ble (bleomycin), Adr (Adriamycin), Epi (epipodophyllotoxin/etoposide), Cycl (cyclosporin A), and Vcr (vincristine). (Adapted from Ref. 1.)

Levin *et al*. 1980. *J. Med. Chem.* 23: 682-684

ABC transporters at the blood-brain barrier





3 most common:

- P-glycoprotein (P-gp/ABCB1)

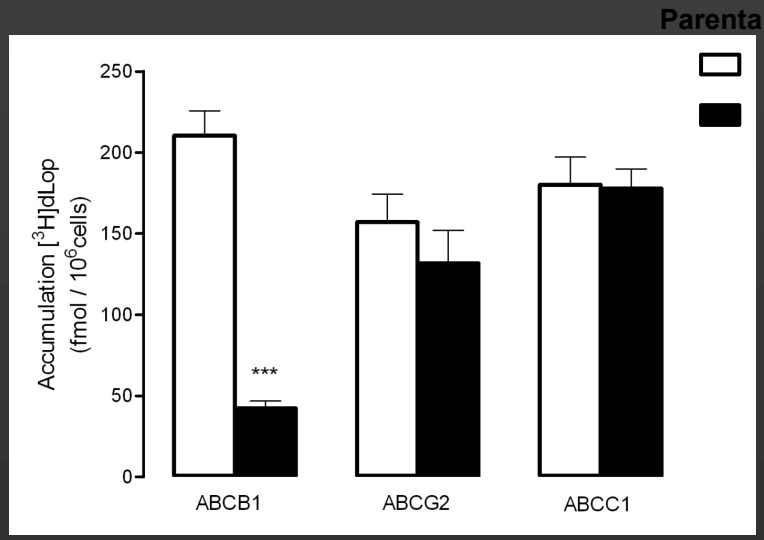
- Multidrug resistance protein (Mrp1/ABCC1)

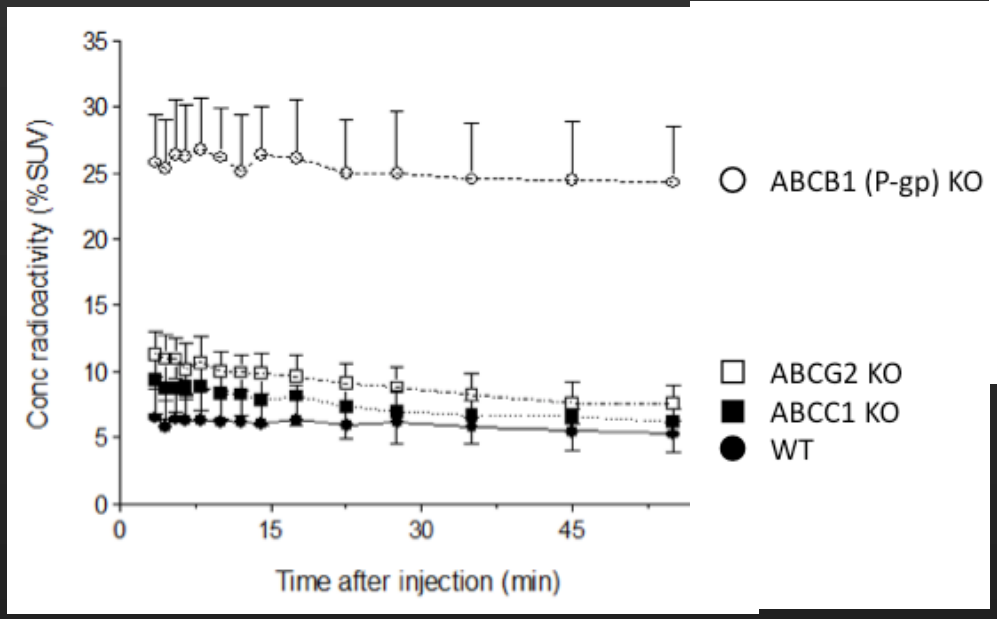
- Breast cancer resistance protein (Bcrp/ABCG2)

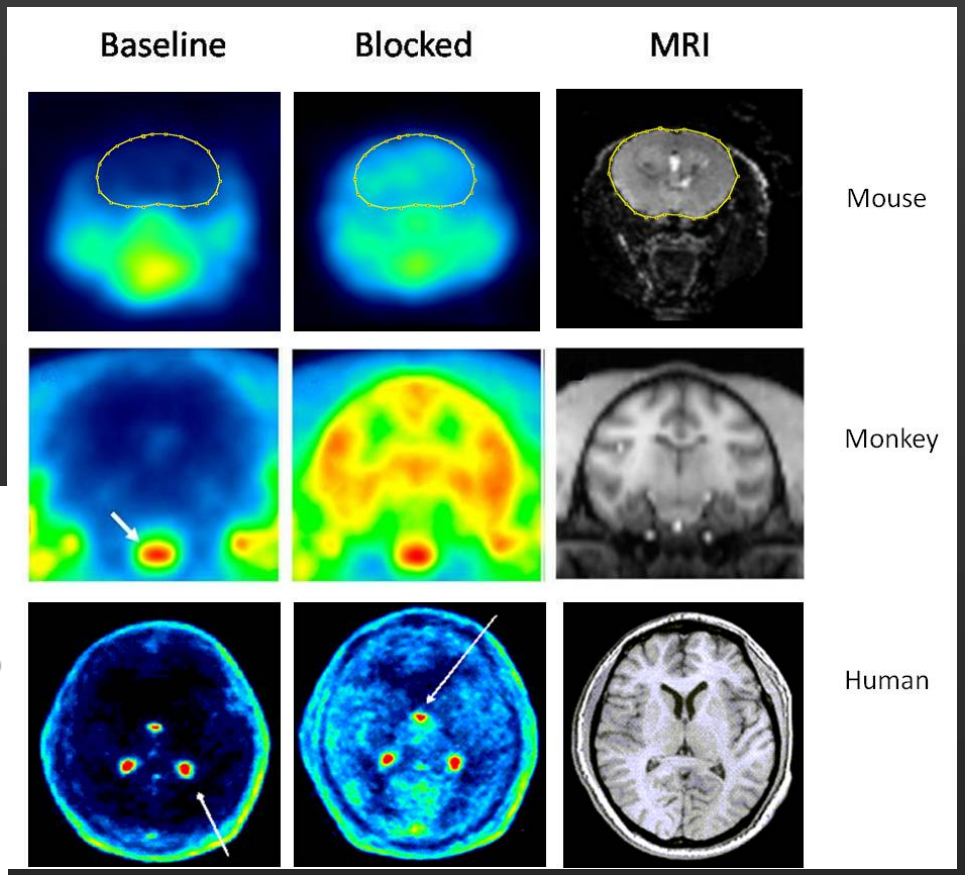
Hall and Pike 2011 *J Nucl Med*

Kannan *et al.* 2009 *Clin Pharm Ther*

dLop is a specific substrate of P-gp







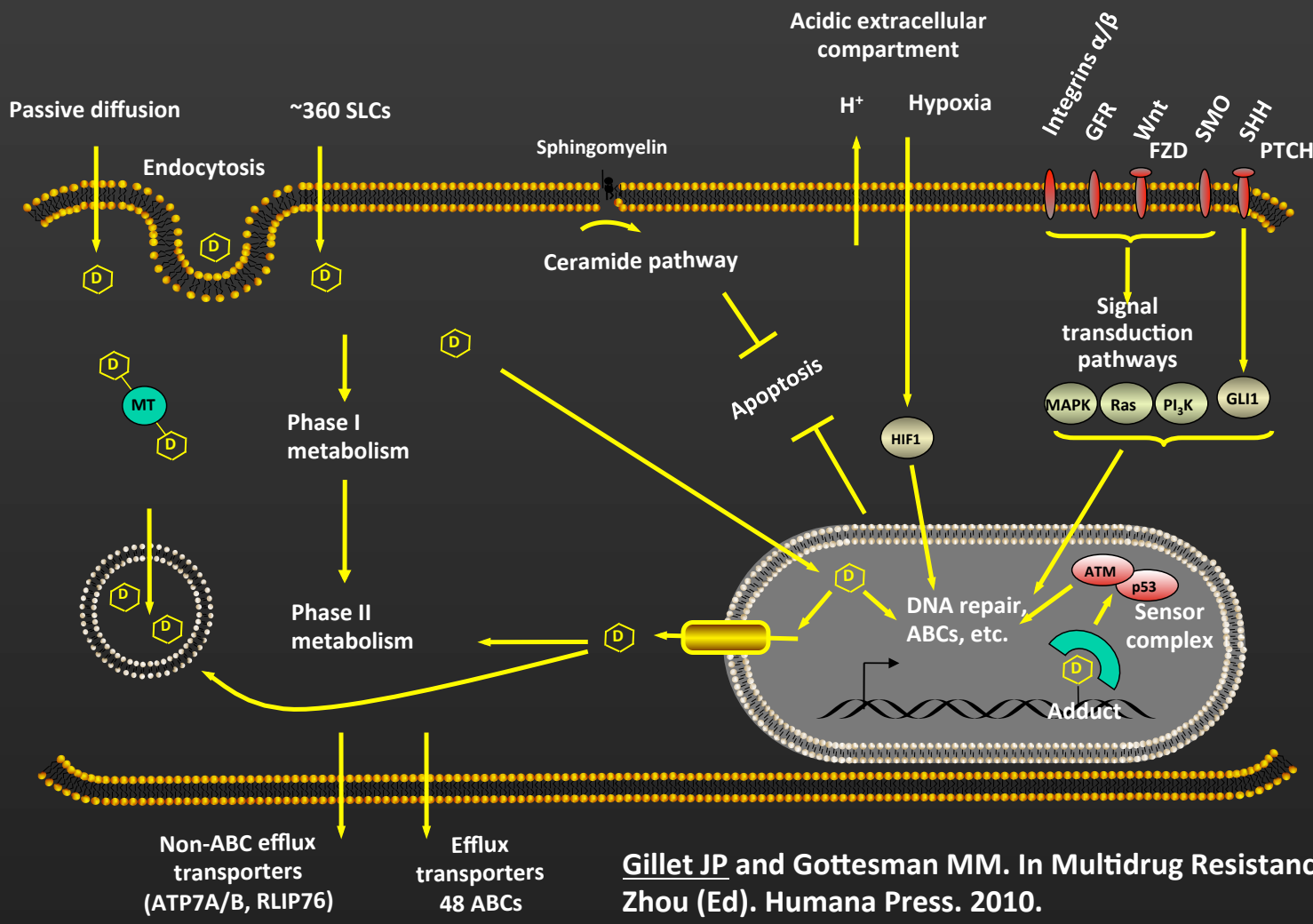
Kannan *et al.* 2010 *Drug Metab Dist*

A likely role for P-glycoprotein (ABCB1) in cancer

- Approximately 50% of human cancers express P-glycoprotein at levels sufficient to confer MDR
- Cancers which acquire expression of P-gp following treatment of the patient include leukemias, myeloma, lymphomas, breast, ovarian cancer; preliminary results with P-gp inhibitors suggest improved response to chemotherapy in some of these patients, but overall response to P-gp inhibitors has been disappointing.
- Cancers which express P-gp at time of diagnosis include colon, kidney, pancreas, liver; these do not respond to P-gp inhibitors alone and have other mechanisms of resistance
- Animal models with human cancer xenografts and BRCA1-driven mouse mammary cancers show role for P-gp in MDR (Pajic et al., Cancer Res. 69, 6396-6404, 2009)

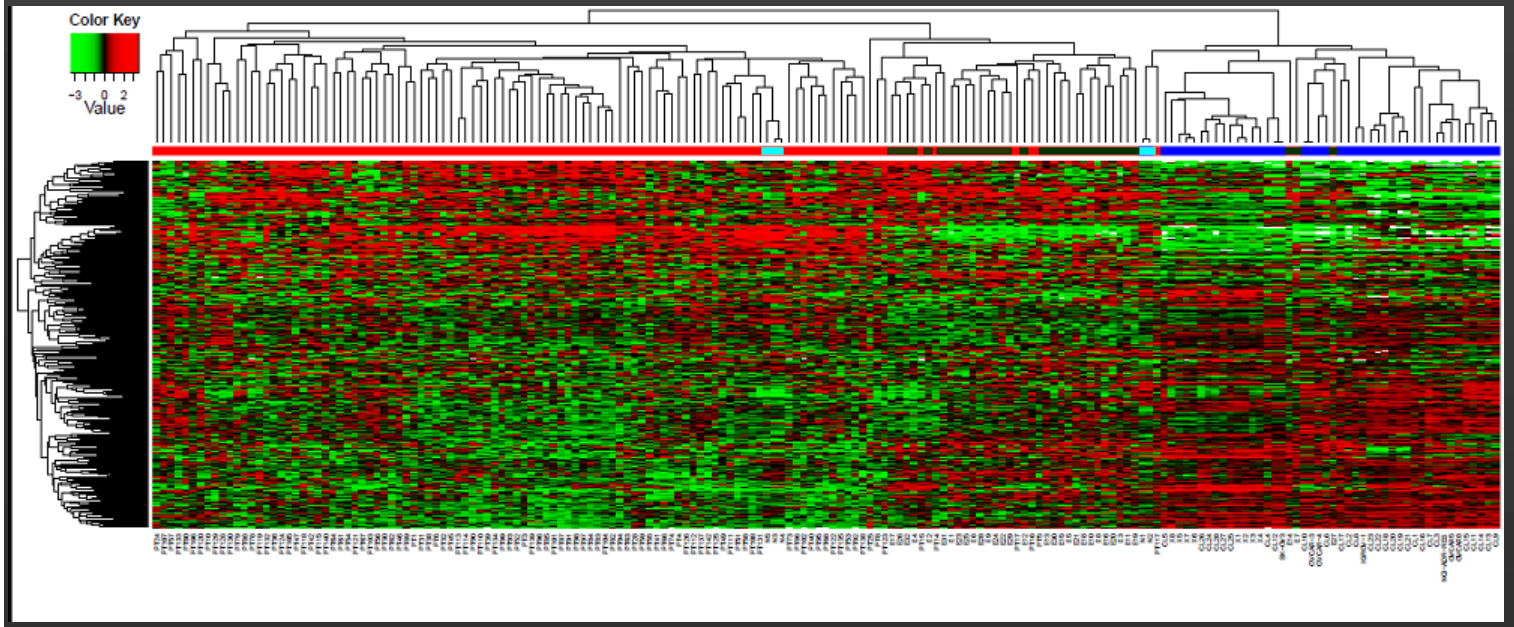
Conclusion: ABCB1 (P-gp) is sufficient, but may not be necessary or the only cause of drug resistance in cancer.

Multiple mechanisms of MD--
the drug resistance transcriptome--
380 genes representing 7 different pathways detected using a dedicated Taqman Low Density Array (TLDA)



Gillet JP and Gottesman MM. In Multidrug Resistance in Cancer, Jun Zhou (Ed). Humana Press. 2010.

Patterns of expression of 380 drug resistance genes in clinical samples and cancer cell lines



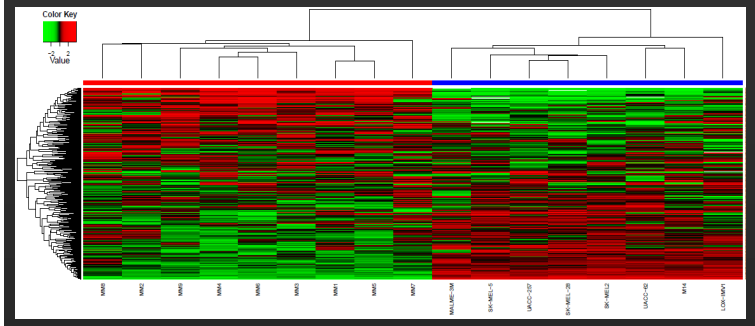
In vivo samples

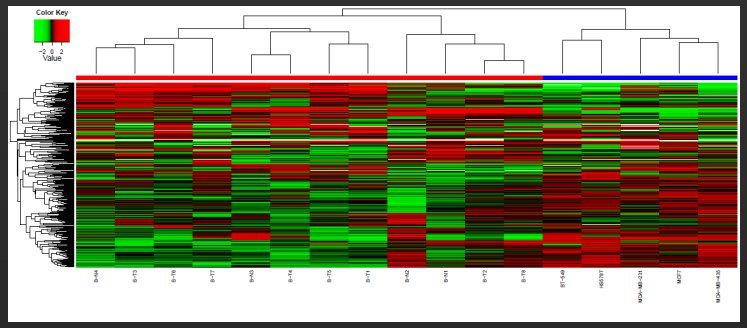
Normal Tissue

Ascites

In vitro samples

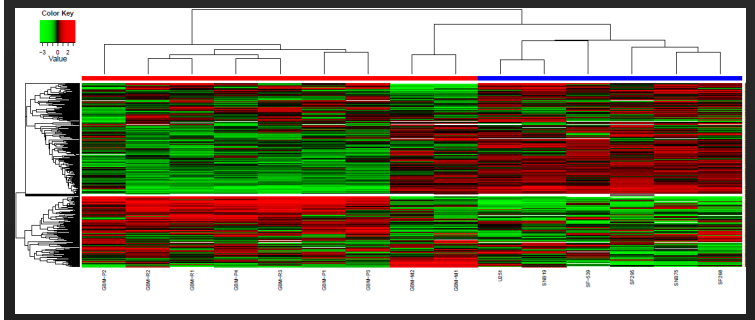
Ovarian cancer

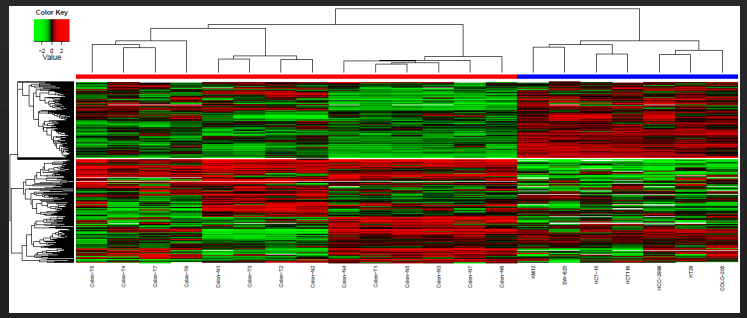




Melanoma

Breast cancer





CNS

Colon cancer

Conclusions from Clinical Studies on Drug Resistance: Cell Culture Models

Current cell culture models for ovarian cancer (and other cancers as well) have patterns of expression of drug resistance genes very different from those of primary cancers; therefore, we need better models for elucidating pathways and contravening drug resistance mechanisms in vivo.

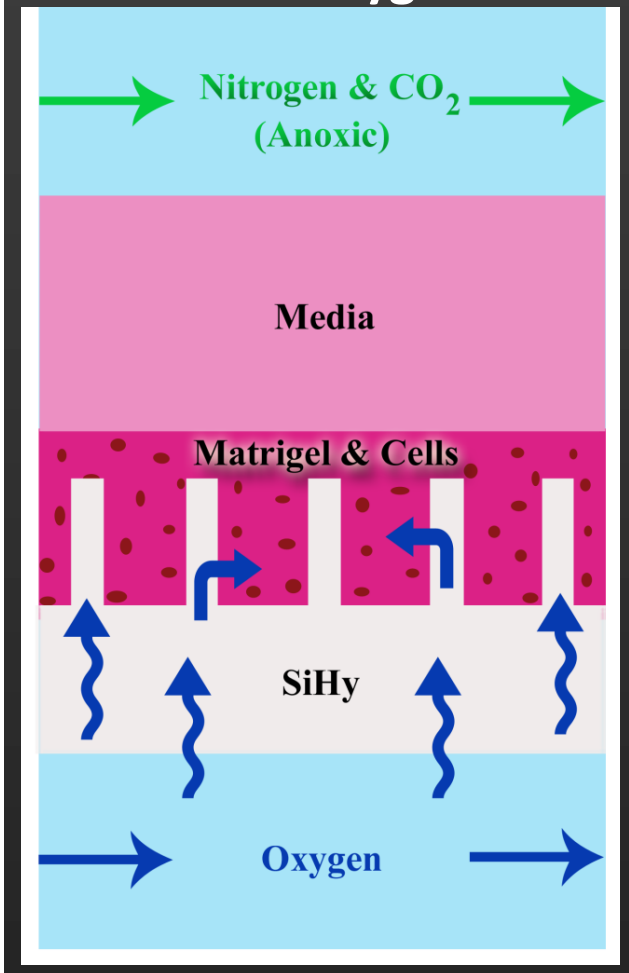
Some reasons why *in vitro* cell culture models do not mimic *in vivo* gene expression

- Cells are selected to grow in tissue culture: survivors may represent a small subset of the original tumors or have mutated to allow *ex vivo* survival
- Culture conditions are different: oxygen tension and gradients, growth factors, monolayer vs. 3D, presence of other cell types
- We force cancers to grow *ex vivo*: normal mitotic index for solid tumors may be <0.1%

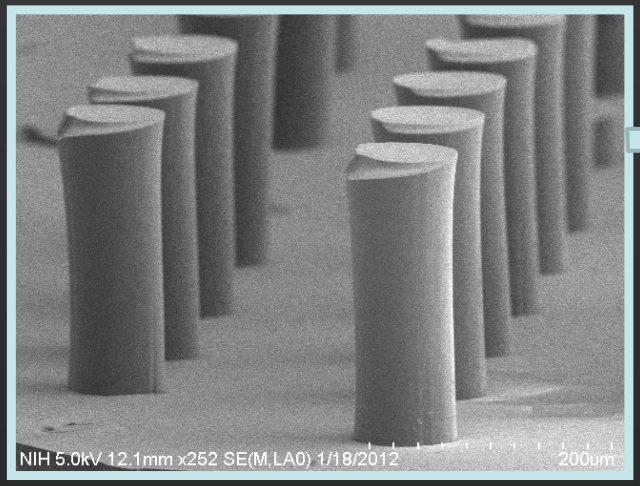
Design and Construction of Bioreactor

(with Ashley Jaeger and Tom Pohida, CIT)

Design concept for mimicking *in vivo* oxygenation

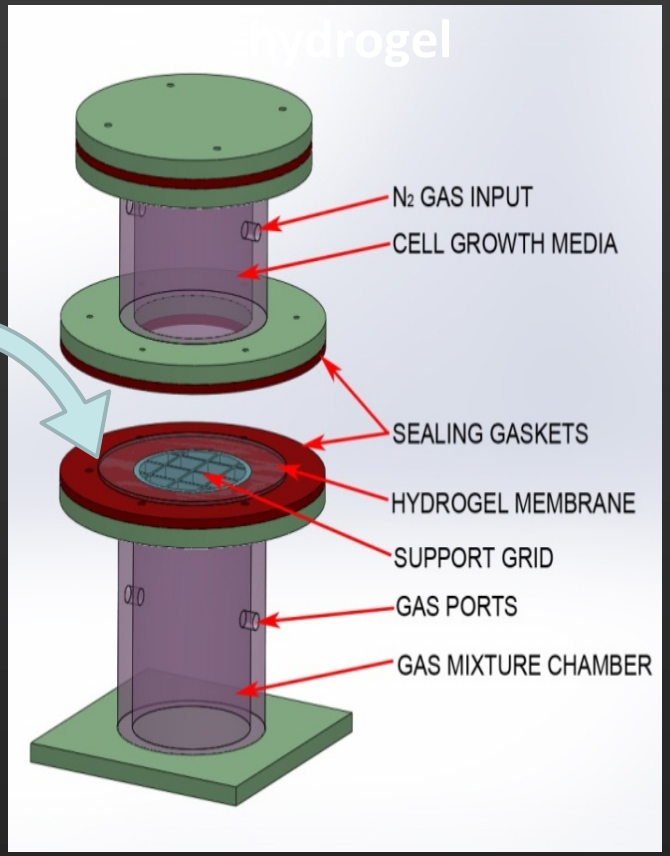


Silicone hydrogel membrane with micropillar structures

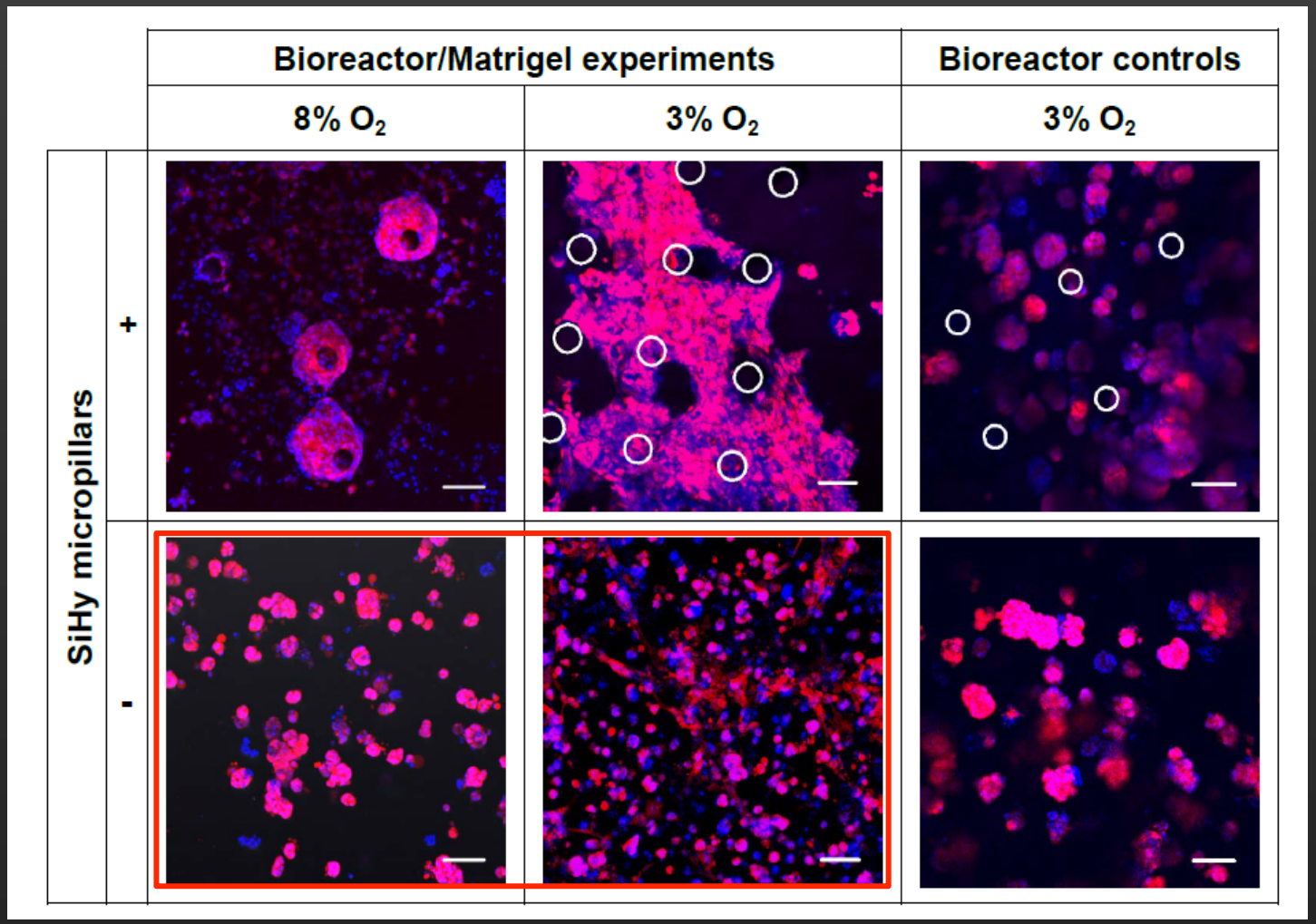


Micropillar diameter range: 25 – 100 µm; Height range: 200 – 250 µm

Bioreactor design for 3-D cell culture on silicone hydrogel

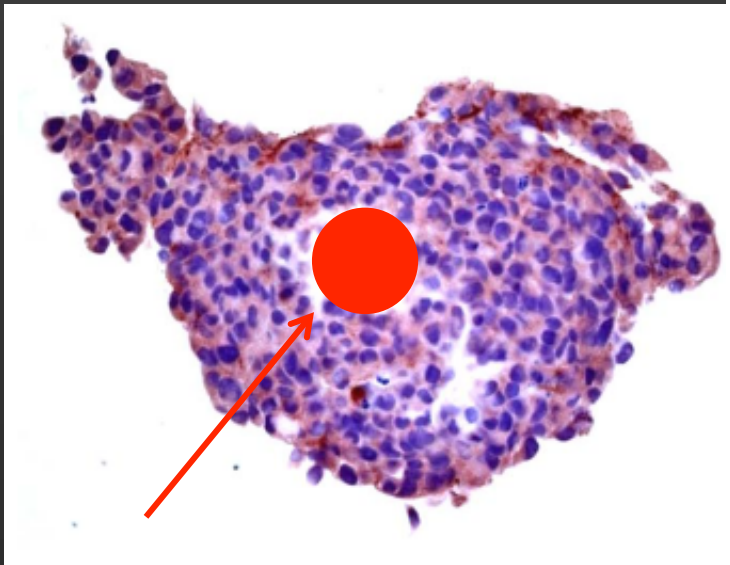


Culture on silicone hydrogel vessel mimetics creates altered growth patterns

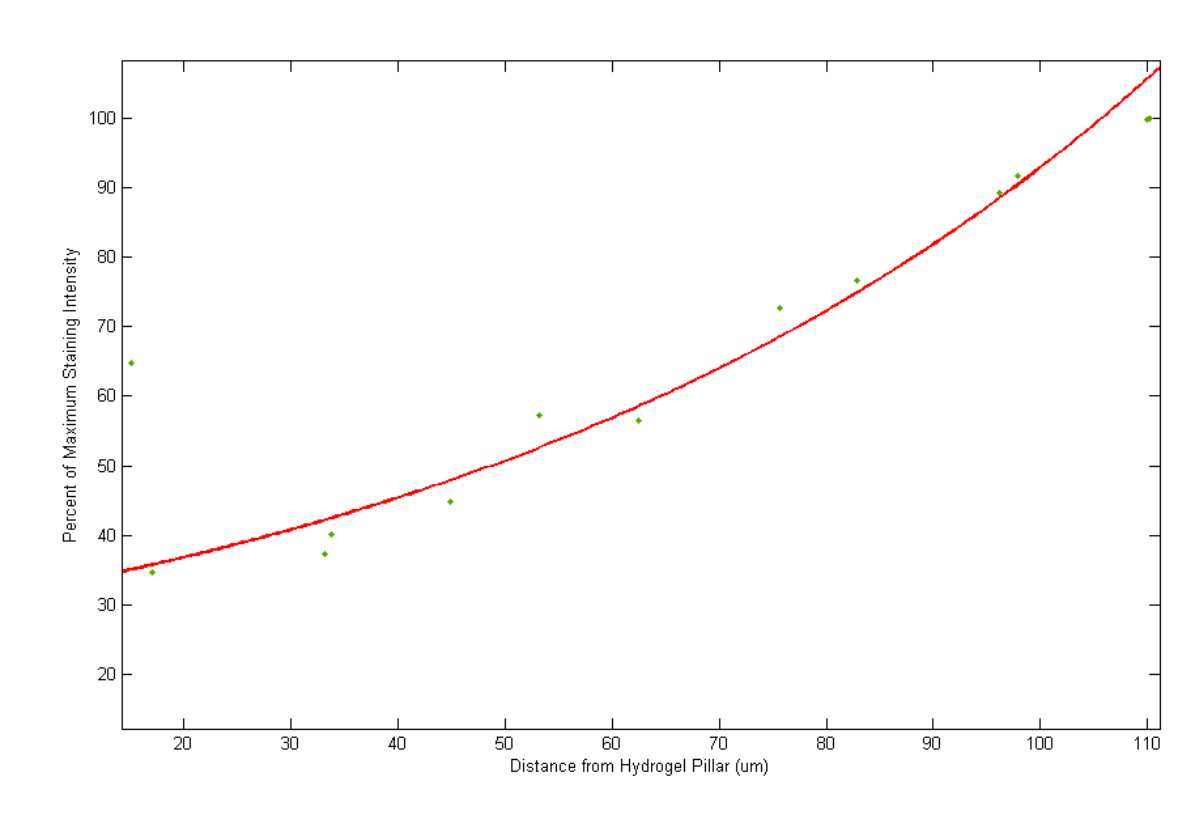


Matrigel controls: Characteristic 3-D culture in basement membrane extract (Matrigel) with OVCAR8-dsRed2 fluorescent cell line. (Scale bar: 100 µm)

Hypoxia gradient in spheroids surrounding micropillars



Location of silicone hydrogel micropillar



Silicone hydrogel culture hypoxic gradient

The gradient obtained by pimonidazole staining was quantified using the MATLAB image processing toolbox and showed a hypoxic drop-off >100 µm from a micropillar.

Cancers used to correlate expression of MDR genes with clinical outcome

- Serous Adenocarcinoma of the Ovary (intrinsic and acquired resistance)
- Hepatoma (mostly intrinsic resistance)
- Acute Myelogenous Leukemia (AML) (mostly acquired resistance)

MDR-linked gene signature for prognosis in ovarian cancer

Genes	Gene Names	p-value	% CV Support
GPX3	Glutathione peroxidase 3	0.0003	100
APC	Adenomatosis polyposis coli / Tumor suppressor	0.0009	100
BAG3	BCL2-associated athanogene 3	0.0012	100
S100A10	Calcium-binding protein S100A10	0.0013	100
EGFR	Epidermal growth factor receptor	0.0023	98.75
ITGAE	Integrin, alpha E	0.0038	98.75
MAPK3	Mitogen-activated protein kinase 3	0.0053	93.75
TAP1/ABCB2	Antigen peptide transporter 2	0.0056	96.25
BNIP3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3	0.0063	90
MMP9	Matrix Metallopeptidase 9	0.0074	86.25
FASLG	Fas ligand	0.0085	66.25

*% CV support: percent of times when the gene was used in the predictor for a leave-one-out cross-validation procedure

Conclusions from Clinical Studies on Drug Resistance: Ovarian Cancer

- It is possible to find a subset of drug resistance genes that improves prediction of poor response to chemotherapy in ovarian cancer; whether manipulation of some or all of these mechanisms of resistance in vivo will improve response to chemotherapy remains to be seen.
- One reasonable hypothesis from these results is that intrinsic drug resistance in ovarian cancer is multifactorial since no single drug resistance mechanism is dominant in predicting poor outcome. Another possible interpretation is that there are individual resistance mechanisms (e.g. P-gp) in subsets of heterogeneous cancers that do not rise to statistical significance. A third is that cancers with different gene expression patterns arise from different origins.

Conclusions from Clinical Studies on Drug Resistance: Hepatoma

- In hepatoma, there is a 45 MDR gene signature that distinguishes poor prognosis from better prognosis.
- This signature has been independently confirmed in a separate set of hepatomas
- These data suggest either two different cells of origin of hepatoma with different signatures, or different pathways by which hepatoma develops

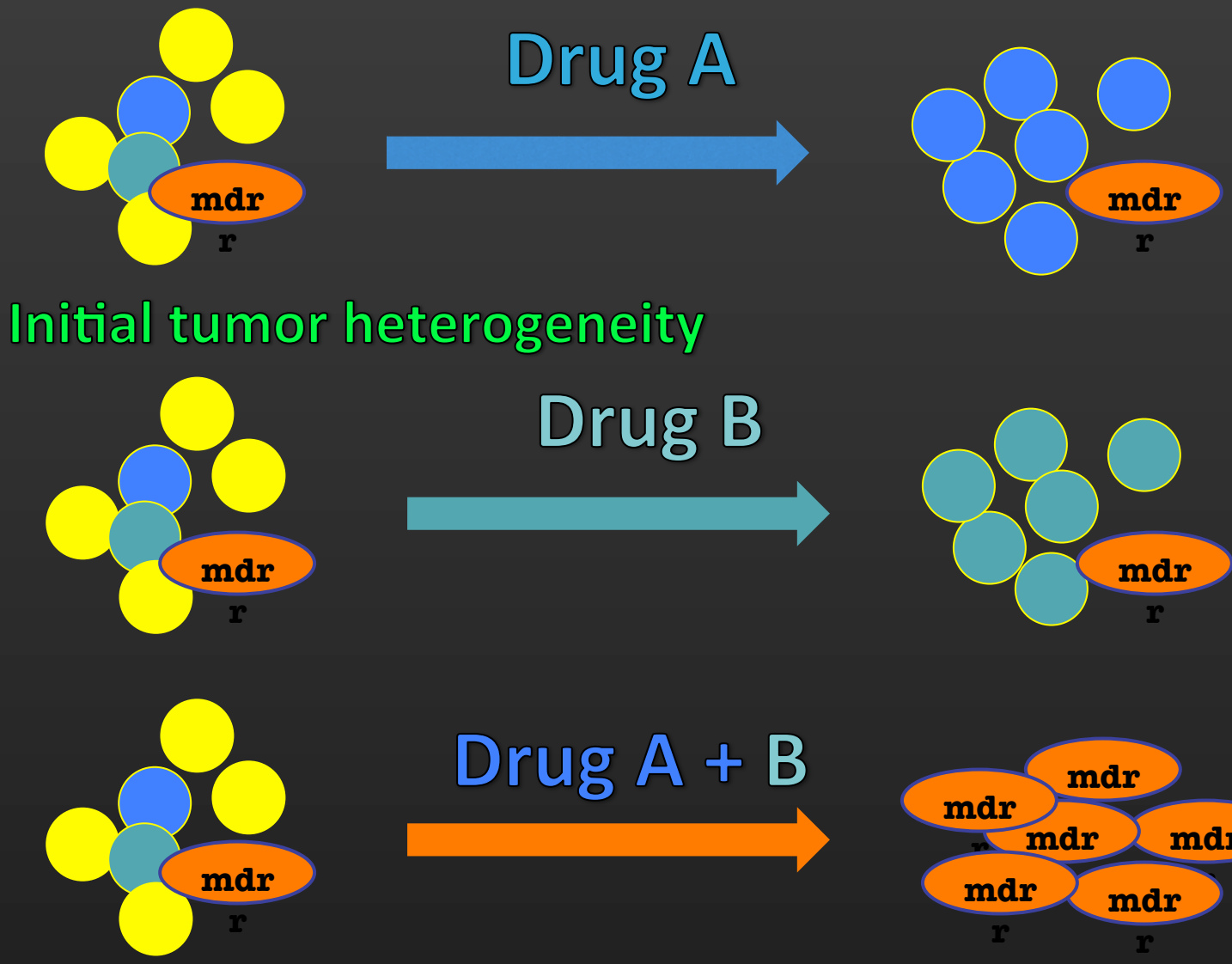
Conclusions from Clinical Studies on Drug Resistance: AML

- Although P-gp expression has been shown to correlate with poor response to chemotherapy in AML, a more detailed analysis shows that multidrug resistance mechanisms are specific to each patient with AML.

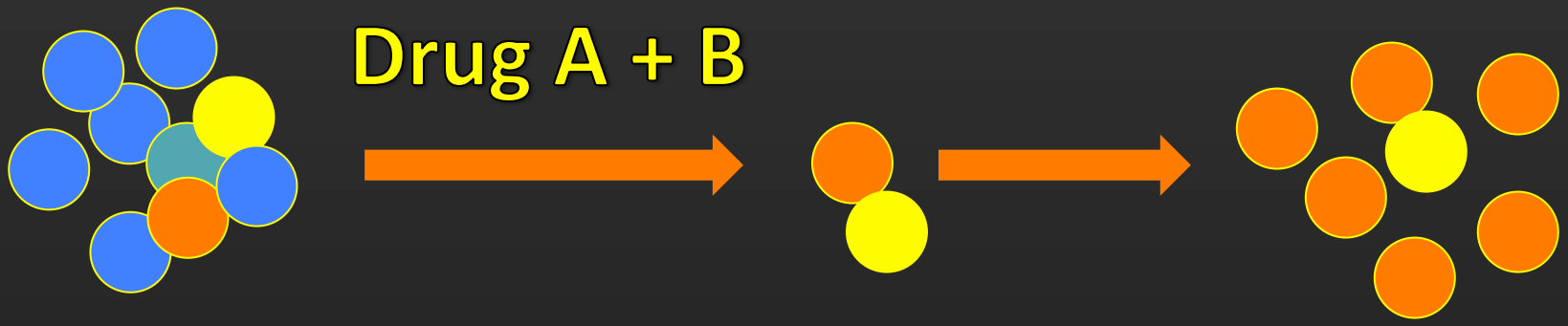
- ABCB1 (P-gp) is not the only anthracycline or Vinca alkaloid transporter expressed in AML

- Relapsed samples of AML overexpress a wide panel of multidrug transporters, suggesting the basis of resistance may be somewhat different in each cancer.

Model To Account for Clinical Results: Acquired Resistance (ovarian cancer, AML)



Model To Account for Clinical Results: Intrinsic Resistance in Ovarian Cancer, Hepatoma



Two Initial tumor types (Different origins or different pathways to malignancy)

Final Thought

Natural product anti-cancer drugs have evolved over billions of years to kill competing cells and organisms. They target multiple pathways in cells that have also evolved over time to preserve life in the face of extreme environmental conditions. Targeted drugs have not been more successful in curing cancer because they target only single pathways.

Acknowledgements

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- James Greene, UMD

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